fields of view showing an intermediate to high amount of extracellular iron aggregates compared to ferucarbotran in both cell-types (11.1 - 19.3% vs 47.6 - 57.2%) (Figure 1C, D).
Conclusions: We have shown that ferumoxides-protamine is more effective and specific compared to ferucarbotran for labelling of hBMSCs and chondrocytes using SPIO. In order to verify the effectiveness and safety of cell therapies for cartilage repair, this provides major opportunities for clinically applicable cell tracking in an intra-articular environment.

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FEASIBILITY OF SCAFFOLD-FREE AGGREGATES OF YOUNG HUMAN CHONDROCYTES FOR CARTILAGE REGENERATION IN *IN VITRO* AND *EX VIVO* CARTILAGE EVALUATION MODEL

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Purpose: The efficiency of cartilage regeneration is thought to be highly affected by the chemical and mechanical properties of engineered cartilage before implantation. The physicochemical properties of cartilage implants go through the biological maturation and structural remodeling after implantation as well as during the engineering process.
The purpose of present study was to evaluate the maturity and remodeling of a scaffold-free chondrocytes aggregates from young human sources *in vitro* and *ex vivo* using explants of human femoral condyle.
Methods: *1. Cell source and the scaffold-free system:* Human chondrocytes were isolated from the polydactyly tissues from 3 patients (1or 2 years old) and cultured in our scaffold-free cartilage fabrication system, previously described. The fabricated scaffold-free chondrocytes aggregate was cultivated in vitro for 1, 7, 21, and 35 days before various assays. *2.* Ex vivo *cartilage tissue regeneration system:* Full thickness osteochondral defect (d = 5 mm) was created in the explant of human lateral femoral condyle from 5 patients undertaken joint replacement surgery (age range of 64-74 years). The defects of explants were left empty as a negative control (group 1) or filled with the scaffold-free chondrocytes aggregate cultured for 7 days in vitro by press-fitting method (group 2). The autologous osteochondral tissue (AOTS) was used as a positive control group (group 3). The explants with each construct were implanted subcutaneously into the back of nude mice and incubated for 4 weeks. *3. Evaluation of the fabricated constructs* in vitro *and* ex vivo: The scaffold-free chondrocytes aggregate fabricated *in vitro* and *ex vivo* were evaluated by macroscopic observation, quantitative biochemical analysis, histological assays and mechanical test. The ratio between the contents of chondroitin sulfate-4 and -6 were also evaluated.
Results: In the static culture system in vitro, the scaffold-free chondrocyte aggregate formed a white disk-shape construct with rather irregular surface at all time points until 35 days. However, the amount of GAG and collagen increased until 21 days and reached plateau afterward. When the scaffold-free chondrocyte aggregate cultured for 7 days in vitro was implanted into the ex vivo model, both of the contents of biochemical components (DNA, GAG and collagen) and mechanical strength were significantly higher than those cultured in vitro for 35 days and were more than 50% of those of native cartilage tissues. The histochemical observations showed positive staining of sulfated proteoglycans and type II collagen although they were not as strong as those of native tissues. In the Safranin-O staining, it appeared integrated with host tissues better than AOTS group did. Furthermore a little bit of collagen fiber formation was also observed in the Sirius red staining.
Conclusions: In the present study, we showed that the scaffold-free aggregates of human polydyctyly chondrocytes were fabricated well into the cartilage-like structure *in vitro* and *ex vivo* in the histochemcial, biochemical and mechanical analyses. This result suggests that our scaffold-free fabrication system using young human chondrocytes could be a potential treatment to regenerate the cartilage defect in human. To our knowledge, this is the first report to apply chondrocytes from polydactyly patients to the scaffold-free system for cartilage regeneration.

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BONE MARROW DERIVED CELL CHARACTERIZATION FOR THE TREATMENT OF EARLY OSTEOARTHRITIS LESIONS BY A SINGLE STEP PROCEDURE

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Purpose: In the past years attention has been focused on the promising technology of cell-assisted repair that propose the use of autologous chondrocytes for the treatment of cartilage lesions due to traumatic events. The opportunity to enlarge the indications to the treatment of early degenerative changes in OA patients is very intriguing. Even if the use of MSCs as a pure cell lineage has been advocated for cartilage repair to overcome problems associated with donor-site morbidity, cell de-differentiation and the limited lifespan of chondrocytes, the opportunity to implant concentrated bone marrow in a single operative procedure is of great importance. The rationale lies in the key role of bone marrow microenvironment (or"niche") which contains not only stem cells and precursors cells as a source of regeneration tissue, but also accessory cells that support angiogenesis and vasculogenesis by producting several growth factors and cytokines.
Aim of the research was directed toward the conduction of an in vitro study to characterize human concentrated bone marrow-derived cells (BMDc) and their ability to differentiate into cartilage and bone.
Methods: Human bone marrow was obtained from the posterior iliac crest of patients underwent autologous cell transplantation for osteochondral defects and directly concentrated in operative room by a cell separator. To characterize BMDc a series of cellular markers has been evaluated using monoclonal antibodies anti- CD45, CD34, CD54, CD63, CD90, CD105, CD106, CD146, CD271 by flow cytometric analysis. The ability of the cells to form colonies was analized by CFU-F test. The potentiality of concentrate cells to differentiate into chondrogenic and osteogenic lineages was analyzed after culturing the cells up to 28 days in differentiating media and evaluated by Alcian Blue and Alizarin red S staining.
Results: BMDc expressed CD34, CD45, CD54 and CD63 at the same levels as isolated MSCs, while CD90, CD105, CD106, CD146 and CD271 were highly expressed in BMDc compared to MSCs. BMDc were able to form colonies and once they are cultured in chondrogenic medium added with TGFβ were positive for Alcian Blue staining on day 28 showing the presence of extracellular matrix specific molecules. At the same experimental time, a lot of colonies positive to Alizarin red S in BMDc cultured in osteogenic medium were found. Calcium precipitates were evident inside the colonies.
Conclusions: Bone and cartilage defects are common features of joint diseases in OA. Despite progress in orthopaedic surgery, the repair of these tissues is a major challenge as they do not spontaneously heal. The chance to use autologous cell transplantation in degenerative lesions in OA patients represents an important therapeutical strategy for the disease. Patients could benefit of such